REMARKS

Status of the application

Prior to entry of this amendment, claims 1-18, 21 and 23 are pending and stand rejected in the application. With entry of this amendment, claims 3 and 18 have been canceled, claims 1, 2, 4, 6-9, 15, 17, and 21 have been amended. Therefore, claims 1-2, 4-17, 21, and 23 are now pending in the application.

Support for amendments to claim 1(d) and claim 17 (d) is found in the specification, e.g., the original claim 3. The recitation of "fully or partially sequenced at 10 or more different locations" in claims 7-9 has support in the specification, e.g., at page 12, lines 22-26. No new matter have been introduced by the claim amendments.

Claim amendments are for purposes of improved clarity or consistency of claim language unless otherwise noted. No claim amendment should be construed as an acquiescence in any ground of rejection. The following remarks address issues raised in the Office Action.

Claim objections

The Examiner objected to the spelling of "hybridised" or "immobolised" in the claims. The claims have been amended as suggested by the Examiner. Accordingly, the objections have been obviated.

Claim rejection under 35 U.S.C. § 112, second paragraph

Claims 1-17 were rejected as allegedly lacking proper antecedent basis for the recitation of "the appropriate position" in claims 1 and 17. The Examiner suggested that the word "the" be replaced with "a". In response, Applicants submit that in the context of primer extension, there can only be one "appropriate position" (i.e., the position next to be extended). Therefore, Applicants believe that the word "the" is correctly used and is preferable to "a". Accordingly, withdrawal of the instant rejection is respectfully requested.

The Examiner alleged that claims 1-17 are indefinite for being incomplete because claim 1 lacks a step in which nucleic acid molecules are sequenced. Claim 1 and 17 have been amended to recite the alleged missing step. The Examiner also says that it is unclear

"how claim 1 operates with one nucleotide 'sequences' a nucleic acid." Applicants assume that the Examiner is referring to the recitation in claim 1 that the plurality of nucleic acid molecules have the same "sequences". Applicants have amended claim 1 by replacing "sequences" in steps (a) and (b) with "sequence". Therefore, Applicants believe the rejection is obviated.

The Examiner rejected claim 2 as being *non sequitur* to claim 1 because it has no recited relationship to the claimed method. In response, Applicants have amended claim 2 by reciting more clearly that claim 2 depends from claim 1 and comprises an extra step relative to claim 1. Therefore, the rejection is overcome.

Claim 4 was rejected for the recitation of "e.g.". Deletion of such wording in the amended claim moots the rejection.

Claims 7-9 were rejected on the ground that the claims, by reciting a "use" of the method, are unclear as to whether the numerous different nucleic acid molecules are sequenced. The claims have been amended to correct such alleged indefiniteness.

As suggested by the Examiner, the recitation of "incorporated" in claim 21 has been replaced with "incorporation". Therefore, rejections to claim 21 and dependent claim 23 have been overcome.

Rejection of claims 1, 2, 4-17, 21, and 23 and under 35 U.S.C. § 103

Claims 1, 2, 4-17, 21, and 23 were rejected under 35 USC 103(a) as being unpatentable over Rabani et al. (International Application No. WO 96/27025) in view of Brenner (U.S. Patent No. 5,863,722). In maintaining the rejection, the Examiner takes the view that Rabani discloses all the elements of claim 1 of the subject application except that Rabani does not teach sequencing diverse populations of identical molecules. The Examiner also says that Brenner teaches sequencing nucleic acid molecules comprising providing populations of identical single-stranded nucleic acid molecules having the same sequence as one another. The Examiner then concludes that it would have been obvious to a skilled person to modify the method of Rabani with the teachings of Brenner to obtain the claimed invention, because the skilled person would have been motivated to sequence diverse populations of identical nucleic acid molecules with a reasonable expectation of success for the benefit of

increasing large-scale DNA sequencing and lowering of sequencing cost in a significant manner as taught by Brenner. This rejection is respectfully traversed for the reasons stated below.

1. Claim 1 and dependent claims 2 and 4-16 are nonobvious over Rabani et al. in view of Brenner et al.

i). Rabani teaches away from the presently claimed method

The approach set out in claim 1 of the subject application relies upon detecting whether or not a label has been incorporated at first and second locations, each location comprising a plurality of molecules having the same sequence as one another and the sequence of the molecules at the first location being different from that of molecules at the second location. Thus, if a label is detected at a given location it provides a cumulative signal arising because the label is incorporated into a plurality of identical extended primers that are present at said location.

By contrast, Rabani goes in completely the opposite direction from the method set out in present claim 1. It is concerned with the parallel examination of individual molecules (rather than populations of identical molecules provided in defined regions). This is clear from the following passage from Rabani et al:

> The subject application approaches the vastness of biological complexity through massive parallelism, which may conveniently be attained through various single molecule examination (SME) methods variously referred to heretofore as single molecule detection (SMD), single molecule visualization (SMV) and single molecule spectroscopy (SMS) techniques. Used within appropriate procedures, single molecule examination methods can enable molecular parallelism.[page 5, lines 27 to 33 of Rabani; emphasis added]

Rabani contrasts prior art methods relying upon sequencing populations of identical molecules with its "distinct single molecule regime" and list various alleged "substantial advantages". These advantages are said to be "important in the competitiveness of these present methods over conventional polynucleotide sequencing methods" (see, e.g.,

page 7, lines 38-41). The basic approach taken by Rabani and the alleged advantages are illustrated in the following paragraph of Rabani:

> In contrast to the previously disclosed base-addition sequencing schemes, the sequence determination applications of the subject application enjoys substantial advantages deriving from sample manipulation in the single-molecule-regime. Working instead in the distinct single-molecule-regime rather than with populations of identical molecules provides substantial advantages of parallelism, facility of use and implementation (including automated implementation,) and operability. Among these are unanticipated advantages: (1) because a single molecule is necessarily monodisperse, failure of a molecule to undergo addition in a cycle does not cause a loss of sample monodispersion (i.e. lead to uneven sample molecules dispersity or polydispersion); such addition failure is unproblematic when single molecules are examined individually because it is readily detected and account for in data analysis; in contrast, samples comprising multiple identical molecules may thus take on non-identical lengths, complicating data collection and analysis; (2) samples comprising a plurality of individually distinct single molecules (species) may be handled unitarily without requiring any handling measures to keep distinct molecules apart, providing a large reduction in manipulations required on a per-species basis and not requiring the use of many separate, parallel fluid handling steps or means; (3) inadvertent multiple base additions are more readily detected and their extent is more readily quantified because these changes in quantity are large compared to the signal expected from the incorporation of a single base (i.e. single label) into a single molecular species; (4) deprotection or delabeling failures may also be readily detected and noted for the correct single molecule, such that addition failure, the presence of a label, or overlabeling in the subsequent cycle may be correctly interpreted (according to the unlabeling and single stepping methods used in a particular embodiment.) These advantages are expected to be important in the competitiveness of these present methods over conventional polynucleotide sequencing methods." [page 7, line 35 to page 8, line 23; emphasis added]

In addition to the alleged advantages, Rabani also emphasizes the need to keep labelled molecules relatively far apart to avoid "unacceptable proximity", whereby a label from one molecule could interfere with determining whether or not a label has been incorporated into an adjacent molecule (see, e.g. page 13, line 33 to page 14, line 29 of Rabani et al.). This is in complete contrast to the subject application which emphasizes desirability of having very

large numbers of identical template molecules at a single location (see, e.g., page 11, lines 4 to 6 of the subject specification). According to the Rabani method, identical molecules in Rabani et al. method cannot be present at very high densities within a given location because sample molecules must be located on a surface at a "convenient density"(page 42, line 4). Thus, very sensitive microscopic detection techniques are also needed in Rabani so that detection is at the level of individual labels (see e.g. page 42, lines 32 to 35 of Rabani), rather than being at the level of a cumulative signal arising from many labels being incorporated at a given location.

From the above discussion, it is readily apparent that a skilled person, by following the teaching of Rabani would not consider using a different approach from single molecule sequencing. Such an attempt would be expected to result in a loss of the alleged advantages emphasized in Rabani. As such, Applicants submit that disclosure of Rabani et al. expressly teaches away from the method recited in present claim 1.

ii). Rabani et al. requires a removal step that is not required in the presently claimed method.

A further non-obvious feature of claim 1 of the subject application over Rabani is that there is no need to remove or neutralize a label once it has been incorporated by primer extension. For example, Step (e) of present claim 1 recites that "extended primers comprising a plurality of labels are provided". The need for removing/neutralizing label is avoided because a cumulative signal is generated from all of the label incorporated with a plurality of identical molecules at a single location by primer extension. When further rounds of primer extension occur, the signal can increase in a step-wise manner, as shown in Figure 2 of the present application. Because label is incorporated into a plurality of molecules at each primer extension step, the signal produced is relatively intense and can therefore be detected without the need for such sensitive detection apparatus as would be required to analyze single molecules. Furthermore, since sequencing is based upon analyzing locations comprising populations of identical molecules (rather than upon the separate analysis of each individual molecule), molecules can be immobilized at extremely high densities at a given location without worrying about a signal from one of the molecules interfering with a signal from

another of the molecules at said location and without the need for very sensitive detection apparatus.

By contrast, the sequencing method of Rabani requires the removal or neutralization of labels (see e.g. page 6, lines 30 and 31 and page 7, lines 14 and 15 of Rabani). For example, in a preferred embodiment, Rabani indicates that it is necessary to check that label is removed after each labeling and detection step of the preferred embodiment (see page 42, lines 35 to 41 of Rabani).

iii). Brenner et al. does not provide the suggestion or motivation that leads to the presently claimed methods

Brenner et al is an equivalent of WO96/12039, which is discussed at pages 2 and 3 of the subject specification. Regardless of whether Brenner discusses sequencing populations of identical single-stranded molecules, it does not suggest or motivate one to sequence nucleic acid molecules as the presently claimed methods. First, given the clear teaching of Rabani of " working in the distinct single-molecule-regime rather than with populations of identical molecules", it is clear that a skilled person would not combine the teaching of Rabani with the teaching of Brenner in respect of sequencing populations of identical, single-stranded molecules.

In addition, Brenner is concerned with a "method of sorting polynucleotides" (see the title). The method uses a particular tagging system, whereby oligonucleotide tags are provided which comprise a plurality of subunits selected from minimally cross-hybridizing sets of subunits (see, e.g., Col. 5, lines 43-62 of Brenner). The tags can be used to sort polynucleotides onto the surfaces of microparticles, as explained in the second complete paragraph at column 19 of Brenner. The polynucleotides can then be used for various purposes, including (but not limited to) sequencing.

Further, the sequencing method of Brenner is also very different from that of claim 1 of the subject invention. The preferred sequencing method disclosed in Brenner is illustrated in Figures 1b) and 1c). This is also the sequencing method disclosed in WO95/27080, which is discussed at page 3 of the present application and which claims priority from USSN 08/280441 (referred to at column 16, lines 48 and 49 of Brenner). As can be seen

from Figures 1b) and 1c) of Brenner, the molecule to be sequenced (15) has an overhanging 5' end for one strand and a recessed 3' end for the other strand. The 3' end can be considered to be the end of a primer that is hybridised to the other (longer) strand (having said overhanging 5' end). It is true that the 3' end is initially extended with a labelled nucleotide (11). However the labelled nucleotide is then removed. This is done by a procedure in which a probe is ligated to the molecule to be sequenced so as to provide a restriction site for a restriction enzyme, such as Fok1. Cleavage with the restriction enzyme then removes the labelled nucleotide as well as adjacent nucleotide. Thus the primer is shortened. Further cycles of this procedure result in further shortening of the primer.

Thus, the Brenner sequencing approach teaches away from the present claimed invention which relies upon step-wise primer extension rather than primer shortening. The present claimed method also avoids the need for removing labels from a primer so that extended primers comprising a plurality of labels are provided. Furthermore, it avoids the need for using restriction enzymes (such as Fok1), or for probes, such as the one illustrated in Figure 1b) of Brenner.

Based on the above remarks, Applicants submit that claim 1 and dependent claims 2 and 4-16 are non-obvious over the cited references. Accordingly, withdrawal of the rejections is respectfully requested.

2. Claims 17, 21, and 23 are on-obvious over the cited art

Claims 17 is an independent method claim. However it has most of the features of claim 1, the main difference being that a mixture of labelled and non-labelled nucleotides are used for primer extension in step c). Thus, the above remarks in respect of claim 1 apply equally to rejection of claim 17.

Claim 21 is another independent claim. Unlike claim 1, it does not require a plurality of single-stranded molecules that have the same sequences to be provided at a given location. However, like claim 1, it has the feature that primer extension is used to incorporate a plurality of labels into a nucleic acid strand. As discussed in connection with claim 1, this goes against the teachings of Rabani and Brenner. Furthermore, claim 21 specifies in step e) that incremental label incorporated into a primer is detected. This again goes against the

teachings of Rabani and Brenner of removing label after each cycle of primer extension.

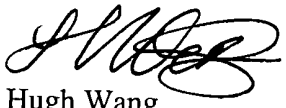
Claim 23 is dependent upon claim 21, and is therefore novel and non-obvious for at least the same reasons that apply to claim 21.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



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